PATENT COOPERATION TREATY

M.H

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 21 January 2000 (21.01.00)	
International application No. PCT/US99/08744	**Applicant's or agent's file reference** 245-52297
International filing date (day/month/year) 20 April 1999 (20.04.99)	**Priority date** (day/month/year) 20 April 1998 (20.04.98)
Applicant ROCKEY, Daniel, D. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 November 1999 (15.11.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Jean-Marc Vivet
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992) 3065299

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 245-52297	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US99/08744	International filing date *(day/month/year)* 20 APRIL 1999	(Earliest) Priority Date *(day/month/year)* 20 APRIL 1998
Applicant THE STATE OF OREGON ACTING BY AND THROUGH THE OREGON STATE BOARD OF HIGHER EDUCATION ON BEHALF OF OREGON STATE UNIVERSITY		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [X] **Unity of invention is lacking** (See Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [X] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08744

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-12 drawn to p242 protein

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08744

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 39/00, 39/118, 49/00; G01N 33/571
US CL :424/9.2, 184.1, 263.1; 435/7.36
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9.2, 184.1, 263.1; 435/7.36

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DYER et al. Analysis of a cation-transporting ATPase of Plasmodium falciparum. Molecular and Biochemical Parasitology. March 1996, Vol. 78, pages 1-12, especially Figure 1.	5, 6
X,P	STEPHENS et al. Genome sequence of an obligate intracellular pathogen of humans: Chlamydia trachomatis. Science. October 1998, Vol. 282, No. 5389, pages 754-759, especially page 754, column 3.	2, 3, 4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
20 JULY 1999

Date of mailing of the international search report
23 AUG 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
RODNEY P. SWARTZ, PH.D.
Telephone No. (703)308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08744

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, CABA, CAPLUS, EMBASE, EMBAL, GENBANK, LIFESCI, MEDLINE, SCISEARCH
search terms: chlamydia, trachomatis, sequence id numbers, vaccine, reticulate body, elementary body, p242 protein

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-12, drawn to p242 *C. tracomatis* protein (SEQ ID NO:2), DNA (SEQ ID NO:1), method of making, and first method of use (vaccination).
Group II, claims 1-12, drawn to TroA *C. tracomatis* protein (SEQ ID NO:4), DNA (SEQ ID NO:3), method of making, and first method of use (vaccination).
Group III, claims 1-12, drawn to TroB *C. tracomatis* protein (SEQ ID NO:6), DNA (SEQ ID NO:5), method of making, and first method of use (vaccination).
Group IV, claims 1-12, drawn to IncB *C. psittaci* protein (SEQ ID NO:10), DNA (SEQ ID NO:9), method of making, and first method of use (vaccination).
Group V, claims 1-12, drawn to IncC *C. psittaci* protein (SEQ ID NO:12), DNA (SEQ ID NO:11), method of making, and first method of use (vaccination).
Group VI, claims 5-12, drawn to IncA *C. psittaci* protein (SEQ ID NO:8)and first method of use (vaccination).
Group VII, claims 5-12, drawn to IncA *C. trachomatis* protein (SEQ ID NO:14)and first method of use (vaccination).
Group VIII, claims 5-12, drawn to IncB *C. trachomatis* protein (SEQ ID NO:16)and first method of use (vaccination).
Group IX, claims 5-12, drawn to IncC *C. trachomatis* protein (SEQ ID NO:18)and first method of use (vaccination).
Group X, claims 13-17, drawn to a second method of use (detection of *Chlamydia*) of p242 *C. trachomatis* protein (SEQ ID NO:2).
Group XI, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of TroA *C. trachomatis* protein (SEQ ID NO:4).
Group XII, claims 13-17, drawn to a second method of use (detection of *Chlamydia*) of TroB *C. trachomatis* protein (SEQ ID NO:6).
Group XIII, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncA *C. psittaci* protein (SEQ ID NO:8).
Group XIV, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncB *C. psittaci* protein (SEQ ID NO:10).
Group XV, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncC *C. psittaci* protein (SEQ ID NO:12).
Group XVI, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncA *C. trachomatis* protein (SEQ ID NO:14).
Group XVII, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncB *C. trachomatis* protein (SEQ ID NO:16).
Group XVIII, claims 13-17, drawn to a second method of use (detection of *Chlamydia*)of IncC *C. trachomatis* protein (SEQ ID NO:18.

Group XIX, claim 18, drawn to a method of treatment of *Chlamydial* infection.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Groups I-IX lack unity with each other as each group is drawn to a structurally (evidenced by different SEQ ID NO) and functionally distinct protein from two different microorganisms (*C. psittaci* and C. trachomatis).
Groups X-XVIII lack unity with each other as each group is drawn to a structurally (evidenced by different SEQ ID NO) and functionally distinct protein from two different microorganisms (*C. psittaci* and C. trachomatis).
Groups I-IX lack unity with Groups X-XVIII because Groups X-XVIII are claiming a second use for Groups I-IX.
Group XIX lacks unity with Groups I-IX because Group XIX is claiming a third use for the proteins of Groups I-IX.
Group XIX lacks unity with Groups X-XVIII because Group XIX is claiming a third use for the proteins of Groups X-XVIII.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08744

Note that PCT Rule 13 does not provide for multiple products or methods within a single application. (See 37 CRF 1.475(d)).

PATENT COOPERATION TREATY

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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: PAULA A. DEGRANDIS
KLARQUIST, SPARKMAN, CAMPBELL, LEIGH & WHINSTON, LLP
ONE WORLD TRADE CENTER, SUITE 1600
121 SW SALMON STREET
PORTLAND, OR 97204

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	14 APR 2000
Applicant's or agent's file reference: 245-52297	REPLY DUE within ONE months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/08744	20 APRIL 1999	20 APRIL 1998

International Patent Classification (IPC) or both national classification and IPC
IPC(7): A61K 39/00, 39/118, 49/00; G01N 33/571 and US Cl.: 424/9.2, 184.1, 263.1; 435/7.36

Applicant
THE STATE OF OREGON ACTING BY AND THROUGH THE OREGON STATE BOARD OF HIGHER EDUCATION ON BEHALF OF OREGON STATE UNIVERSITY

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [X] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

DOCKETED FOR: 5.14.00
ATTORNEY ✓
CLIENT ✓
BOOK ✓
DRAWER ✓
FKPR
ANN. SVE

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 20 AUGUST 2000.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	RODNEY P. SWARTZ, PH.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/408 (cover sheet) (January 1994)★

WRITTEN OPINION

International application No.
PCT/US99/08744

I. Basis of the opinion

1. This opinion has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

[X] the international application as originally filed.

[X] the description, pages (See Attached), as originally filed.
pages _____, filed with the demand.
pages _____, filed with the letter of _____.

[X] the claims, Nos. (See Attached), as originally filed.
Nos. _____, as amended under Article 19.
Nos. _____, filed with the demand.
Nos. _____, filed with the letter of _____.

[X] the drawings, sheets/~~fig~~ (See Attached), as originally filed.
sheets/~~fig~~ _____, filed with the demand.
sheets/~~fig~~ _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

3. [ ] This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:
NONE

WRITTEN OPINION

International application No.
PCT/US99/08744

III. Non-establishment f pini n with regard t novelty, inventiv st p and industrial applicability

The question whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-12 drawn to proteins other than p242, 13-18

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. (See Attached).

WRITTEN OPINION

International application No.
PCT/US99/08744

V. Reasoned statement under Rule 66.2(a)(ii) with regard to n velty, inventiv step or industrial applicability; citati ns and xplanations supp rting such stat ment

1. STATEMENT

Novelty (N)	Claims	1-4, 7-12	YES
	Claims	5, 6	NO
Inventive Step (IS)	Claims	1-4, 7-12	YES
	Claims	5, 6	NO
Industrial Applicability (IA)	Claims	1-12	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 5 and 6 lack novelty under PCT Article 33(2) as being anticipated by Dyer et al.

The instant claims are drawn to a composition comprising at least one purified peptide comprising at least 5 contiguous amino acids selected from SEQ ID NO:2. Following a sequence search, it was found that Dyer et al teach a composition which meets the claim limitations, i.e., a peptide composition comprising ≥5 contiguous amino acids selected from SEQ ID NO:2 (Figure 1).

Claims 1-4 and 7-12 drawn to the p242 protein as exemplified by the amino acid sequence SEQ IDNO:2 and the nucleic acid sequence SEQ ID NO:1 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the purified protein p242, or vaccine preparations where the peptide comprises ≥15 contiguous amino acids of SEQ ID NO:2.

---------- NEW CITATIONS ----------

NONE

WRITTEN OPINION

International application No.
PCT/US99/08744

Supplemental Box
(To be used when th space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

I. BASIS OF OPINION:

This opinion has been drawn on the basis of the description,
pages, 1-26 AND SEQUENCE LISTING PAGES 1-19, as originally filed.
pages, NONE, filed with the demand.
and additional amendments:
NONE

This opinion has been drawn on the basis of the claims,
numbers, 1-18, as originally filed.
numbers, NONE, as amended under Article 19.
numbers, NONE, filed with the demand.
and additional amendments:
NONE

This opinion has been drawn on the basis of the drawings,
sheets, NONE, as originally filed.
sheets, NONE, filed with the demand.
and additional amendments:
NONE

III. NON-ESTABLISHMENT OF OPINION:

No international search report has been established for claim numbers claims 1-12 drawn to other than p242 protein, 13-18.

PATENT COOPERATION TREATY

04U / 12 JP

From the INTERNATIONAL SEARCHING AUTHORITY

To: ADAM W. BELL
KLARQUIST, SPARKMAN, CAMPBELL, LEIGH & WHINSTON, LLP
ONE WORLD TRADE CENTER, SUITE 1600
121 SW SALMON STREET
PORTLAND, OR 97204

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 245-52297	Date of Mailing *(day/month/year)* 23 AUG 1999 **FOR FURTHER ACTION** **See paragraphs 1 and 4 below**
International application No. PCT/US99/08744	International filing date *(day/month/year)* 20 APRIL 1999

Applicant
THE STATE OF OREGON ACTING BY AND THROUGH THE OREGON STATE BOARD OF HIGHER EDUCATION ON BEHALF OF OREGON STATE UNIVERSITY

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

DOCKETED FOR: 10·23·99
COMPUTER ✓
CARD —
BOOK ✓
DRAWER —
BKPR —
ANN. SVE —

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Lawrence Yo RODNEY P. SWARTZ, PH.D.
Facsimile No. (703) 305-3230	Telephone No. (703)308-0196

Form PCT/ISA/220 (January 1994)★ *(See notes on a...*

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 39/00, 39/118, 49/00, G01N 33/571 | A1

(11) International Publication Number: **WO 99/53948**

(43) International Publication Date: 28 October 1999 (28.10.99)

(21) International Application Number: PCT/US99/08744

(22) International Filing Date: 20 April 1999 (20.04.99)

(30) Priority Data:

60/082,438	20 April 1998 (20.04.98)	US
60/082,588	21 April 1998 (21.04.98)	US
60/086,450	22 May 1998 (22.05.98)	US

(71) Applicant *(for all designated States except US)*: THE STATE OF OREGON, acting by and through THE OREGON STATE BOARD OF HIGHER EDUCATION, on behalf of OREGON STATE UNIVERSITY [US/US]; Office of Technology Transfer, 312 Kerr Administration Building, Corvallis, OR 97331–2140 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ROCKEY, Daniel, D. [US/US]; 3625 N.W. Jackson Street, Corvallis, OR 97330 (US). BANNANTINE, John, P. [US/US]; 903 Yuma, Ames, IA 50014 (US).

(74) Agent: BELL, Adam, W.; Klarquist, Sparkman, Campbell, Leigh & Whinston, LLP, Suite 1600, One World Trade Center, 121 S.W. Salmon Street, Portland, OR 97204 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CHLAMYDIA PROTEINS AND THEIR USES

(57) Abstract

Certain *Chlamydia* proteins have been found to be infection–specific and to be associated primarily with the vegetative Reticulate Body form of *Chlamydia* rather than with the refractile Elementary Body form of *Chlamydia*. The invention includes a vaccine directed against the Reticulate Body form of *Chlamydia* comprising one or more infection–specific proteins, or fraction thereof; a method of using such a vaccine; a method of production of such a vaccine; a method for detection of infection–specific antibodies in a biological specimen; a method for detection of infection–specific antigens in a biological specimen and a method of using therapeutic agents specifically directed against infection–specific peptides, or the genes that code for such peptides, to treat chlamydial infection. The invention also includes the IncB, and IncC proteins of *C. psittaci*, and nucleotides encoding these proteins, and the TroA, TroB and p242 proteins of *C. trachomatis*, and the nucleotides that encode these polypeptides.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
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KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
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LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
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PT	Portugal
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RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

CHLAMYDIA PROTEINS AND THEIR USES

I. FIELD OF THE INVENTION

The present invention relates to the detection of *Chlamydia* and to the diagnosis, treatment and prevention of *Chlamydia* infections in animals.

II. BACKGROUND

Chlamydiae are obligate intracellular bacterial pathogens with a unique biphasic life cycle. They appear as two distinct cellular types, a small dense cell or elementary body (EB) that is enclosed in a rigid bacterial cell wall, and a larger metabolically active reticulate body (RB). The EB is resistant to physical disruption and is infectious, whereas the RB is more fragile and only exists inside cells. The *Chlamydia* life cycle begins with the attachment of the EB form to the host cell which is followed by endocytosis into a nascent vacuole, also called an "inclusion membrane." After EB attachment and entry, replication of the EB form produces RB forms that continue to grow within the vacuole. By 72 hour post-infection, this growth phase is terminated when the RBs condense, and reorganize back to EBs. The lysis of the host cell results in release of EBs to infect new host cells. The difficulties in working with *Chlamydiae* center on the obligate intracellular requirement for growth and the fact that no adequate genetic engineering methods have been developed for this organism.

The genus *Chlamydia* includes two species that are primarily associated with human disease: *C. trachomatis* and *C. pneumoniae*. *C. trachomatis* causes trachoma, an eye disease that is the leading cause of preventable infectious blindness worldwide with an estimated 500 million cases of active trachoma worldwide. *C. trachomatis* also causes a sexually transmitted chlamydial disease which is very common worldwide. *C. trachomatis* also causes lymphogranuloma venereum, a debilitating systemic disease characterized by lymphatic gland swelling. The most serious sequelae of chlamydial genital infections of females include salpingitis, pelvic inflammatory disease, and ectopic pregnancy. In the US alone, it is estimated that over 4 million new sexually transmitted *C. trachomatis* infections occurred in 1990, leading to over four billion dollars in direct and indirect medical expenses. The World Health Organization estimates that 89 million new cases of genital *Chlamydia* occurred worldwide in 1995 (Peeling and Brunham, 1996).

C. pneumoniae causes respiratory diseases including so called walking pneumonia, a low-grade disease such that the infected person frequently fails to obtain treatment and remains in the community as an active, infectious carrier. *C. pneumoniae* is currently of interest because of its strong epidemiological association with coronary artery disease, and there is also some evidence to link it with multiple sclerosis.

Of the other disease-causing species of *Chlamydia*, *Chlamydia psittaci* and *Chlamydia pecorum* are primarily pathogens of wild and domestic animals, but these species may infect

humans accidentally. *C. psittaci* is acquired through respiratory droplet infection and is considered an occupational health hazard for bird fanciers and poultry workers.

There is tremendous interest in the identification of candidate antigens for protection against chlamydial disease. While a prior infection with *C. trachomatis* will protect against a subsequent challenge by the same strain, indicating a protective component that stimulates the host immune response, most serious chlamydial diseases are exacerbated by an overaggressive anti-chlamydial immune response. Antigens recognized in the context of an infection appear to elicit a protective response whereas immunization with purified, killed (EB form) *Chlamydia* results in an immunopathological response. Therefore for the purposes of vaccine development, one needs to find epitopes that confer protection, but do not contribute to pathology. It is an object of this invention to provide *Chlamydia* polypeptides for use as vaccines that induce a protective immune response without inducing the pathological response caused by the antigens associated with the EB form of *Chlamydia*. Such immunostimulatory peptides will be useful in the treatment, as well as in the diagnosis, detection and prevention of Chlamydial infections.

III. SUMMARY OF THE INVENTION

The present invention includes the use of *Chlamydia* proteins that show enhanced expression in the reticulate body (RB) stage relative to the elementary body (EB) stage of the *Chlamydia* life cycle. These proteins are not present at detectable levels in the EB form using current immunological techniques and are thus said to be "infection-specific." Certain of these infection-specific proteins are found in the inclusion membrane of the infected cell, and so have been termed "Inc" proteins. These include the IncA, IncB, and IncC proteins of *Chlamydia* as described in the present disclosure. The genes that encode the IncA, IncB and IncC proteins are referred to as *incA*, *incB* and *incC* respectively. Other proteins of *Chlamydia* described herein have also been shown by the inventors to be infection-specific, but are not known to be incorporated into the inclusion membrane; these include the p242, TroA, and TroB proteins. The TroA and TroB proteins have been so named because they resemble the Tro proteins of *Treponema pallidum*, which are thought to form part of an ABC transport system.

The inventors have shown that the infection-specific *Chlamydia* proteins of the disclosure are recognized by convalescent antisera (i.e., antisera taken from an animal that has recovered from a *Chlamydia* infection) but are not recognized by antisera against the killed EB form of *Chlamydia*. Thus, the proteins are expressed only during active chlamydial infection and are therefore useful as protective antigens. These infection-specific proteins may be used to confer a protective immune response without inducing a pathological effect. Additionally, immunofluorescence microscopy and immunoblotting with antisera demonstrated that the infection-specific proteins are present in *Chlamydia*-infected HeLa cells, but are undetectable in purified EBs and absent in uninfected HeLa cells.

Immunofluorescense microscopy reveals that IncA, IncB and IncC are localized to the inclusion membrane of infected HeLa cells. Reverse-transcription polymerase chain reactions (RT-PCR), northern hybridization data, and restriction analysis revealed that the *incB and incC* genes are closely linked and transcribed in an operon. RT-PCR, restriction analysis and sequential Southern hybridizations of *incA* then *incC* to the same filter provided evidence that *incA* is separated from the *incB* and *incC* operon by about 110 kb. The *C. trachomatis Tro* genes are not closely linked with the p242 gene.

The present invention includes the nucleotide and amino acid sequences for certain infection-specific proteins from *Chlamydia*. These proteins are p242, TroA, and TroB from *C. trachomatis*, and the IncB, and IncC proteins from *C. psittaci*. The scope of the invention includes fragments of these proteins that may be used in a vaccine preparation or that may be used in a method of detecting *Chlamydia* antibodies. Such fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length. They may even encompass the entire protein.

More specifically, the present invention encompasses the purified infection-specific proteins having amino acid sequences as shown in SEQ ID NOS: 2, 4, 6, 10, and 12, amino acid sequences that differ from such sequences by one or more conservative amino acid substitutions, and amino acid sequences that show at least 75% sequence identity with such amino acid sequences.

Then invention also includes isolated nucleic acid molecules that encode a protein as described in the above paragraph, including isolated nucleic acid molecules with nucleotide sequences as shown in SEQ ID NOS: 1,3, 5, 9, and 11.

The present invention also includes a vaccine or immunostimulatory preparation directed against the reticulate body (RB) form of *Chlamydia* comprising one or more purified infection-specific peptides (or portions or fragments thereof, or peptides showing sequence similarity to a portion of such a peptide). Such peptide fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length, of the sequence shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, or 18. Peptides used in such a vaccine may even encompass the entire purified peptide of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, or 18, a peptide that differs from such a peptide by one or more conservative amino acid substitutions, or a peptide having at least 75% sequence identity with such a peptide. Such vaccine preparations may contain one or more pharmaceutically acceptable excipients, adjuvants or diluents.

The invention additionally encompasses methods for making a vaccine, comprising combining a pharmaceutically acceptable excipient with a peptide described herein. Also included is a method of vaccination comprising administering a vaccine as described herein to a mammal.

The present invention also provides a method for the diagnostic use of the disclosed purified infection-specific peptides, for instance by use in a diagnostic assay to detect the presence of infection-specific antibodies in a medical specimen, in which antibodies bind to the *Chlamydia* peptide and indicate that the subject from which the specimen was removed was previously

exposed to *Chlamydia*. Such a method may comprise: (i) supplying a biological sample, such as blood from an animal, that is suspected to contain infection-specific anti-*Chlamydia* antibody, (ii) contacting the sample with at least one infection-specific *Chlamydia* peptide described herein, such that a reaction between the peptide and the infection-specific anti-*Chlamydia* antibody gives rise to a detectable effect, such as a chromogenic conversion; and (iii) detecting this detectable effect.

The present invention also provides a method of using antibodies that bind specifically with the disclosed proteins for detection of infection-specific *Chlamydia* antigen, indicating the presence of *Chlamydia* in the RB stage as distinct from the EB stage. For instance, the relevant infection-specific antibodies may be used to provide specific binding in an Enzyme Linked Immunosorbant Assay (ELISA) or other immunological assay wherein the antibody F_c portion is linked to a chromogenic, fluorescent or radioactive molecule and the F_{ab} portion specifically interacts with, and binds to, an infection-specific protein. Such a method may comprise: (i) supplying a biological sample from an animal suspected to contain an infection-specific *Chlamydia* antigen, and (ii) contacting the sample with at least one infection-specific anti-*Chlamydia* antibody, such that a reaction between the antibody and the infection-specific *Chlamydia* protein gives rise to a detectable effect; and (iii) detecting this detectable effect.

Other aspects of the present invention include the use of probes and primers derived from the nucleotide sequences that encode infection-specific peptides, to detect the presence of *Chlamydia* nucleic acids in medical specimens. Such probes and primers may be nucleotide fragments, of, for example, 15, 20, 25, 30 or 40 contiguous nucleotides of the sequence shown in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, or 17.

An additional aspect of the invention is a method of treating a *Chlamydia* infection by directing a therapeutic agent against a specific target, where the target is chosen from an infection specific protein of *Chlamydia*, a gene that encodes an infection-specific protein of *Chlamydia,* and an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein the therapeutic agent interacts with said target to affect a reduction in pathology.

These and other aspects of the invention will become more apparent from the following description.

IV. SEQUENCE LISTING

SEQ ID NO:1 shows a nucleic acid sequence encoding the p242 *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:2 shows the amino acid sequence of the p242 *C. trachomatis* protein.

SEQ ID NO:3 shows a nucleic acid sequence encoding the TroA *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:4 shows the amino acid sequence of the TroA *C. trachomatis* protein.

SEQ ID NO:5 shows a nucleic acid sequence encoding the TroB *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:6 shows the amino acid sequence of the TroB *C. trachomatis* protein.

SEQ ID NO:7 shows a nucleic acid sequence encoding the IncA *C. psittaci* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:8 shows the amino acid sequence of the IncA *C. psittaci* protein.

SEQ ID NO:9 shows a nucleic acid sequence encoding the IncB *C. psittaci* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:10 shows the amino acid sequence of the IncB *C. psittaci* protein.

SEQ ID NO:11 shows a nucleic acid sequence encoding the IncC *C. psittaci* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:12 shows the amino acid sequence of the IncC *C. psittaci* protein.

SEQ ID NO:13 shows a nucleic acid sequence encoding the IncA *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:14 shows the amino acid sequence of the IncA *C. trachomatis* protein.

SEQ ID NO:15 shows a nucleic acid sequence encoding the IncB *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:16 shows the amino acid sequence of the IncB *C. trachomatis* protein.

SEQ ID NO:17 shows a nucleic acid sequence encoding the IncC *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:18 shows the amino acid sequence of the IncC *C. trachomatis* protein.

SEQ ID NO:19 shows the upstream oligonucleotide used to amplify the *C. psittaci incC* ORF.

SEQ ID NO:20 shows the downstream oligonucleotide used to amplify the *C. psittaci incC* ORF.

SEQ ID NO:21 shows the upstream oligonucleotide used to amplify the *C. psittaci incB* ORF.

SEQ ID NO:22 shows the downstream oligonucleotide used to amplify the *C. psittaci incB* ORF.

SEQ ID NO:23 shows the upstream oligonucleotide used to amplify the *C. psittaci incA* ORF.

SEQ ID NO:24 shows the downstream oligonucleotide used to amplify the *C. psittaci incA* ORF.

V. DESCRIPTION OF THE INVENTION

A. DEFINITIONS

Particular terms and phrases used herein have the meanings set forth below.

"EB" refers to the Elementary Body, an environmentally refractile and largely metabolically dormant form of *Chlamydia* that is infectious and is presented as a small dense body enclosed by a bacterial cell wall.

"RB" refers to the Reticulate Body, a metabolically active form of *Chlamydia* that is not infectious, and exists only within a host cell, being very fragile, often branched, and appearing larger and less dense that the EB.

"Infection-specific" refers to a protein that shows enhanced expression in the RB form of *Chlamydia* compared to the EB form. Infection-specific proteins are not necessarily absent from the EB form, but they are significantly more common in the RB form than in the EB form.

"infection-specific antibody" is an antibody that binds specifically to an infection-specific protein.

"Biological sample" refers to any sample of biological origin including, but not limited to a blood sample, a plasma sample, a mucosal smear or a tissue sample.

"Isolated" An isolated nucleic acid has been substantially separated or purified away from other nucleic acid sequences in the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA. The term "isolated" thus encompasses nucleic acids purified by standard nucleic acid purification methods. The term also embraces nucleic acids prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

"Probes" and "primers." Nucleic acid probes and primers may readily be prepared based on the nucleic acid sequences provided by this invention. A "probe" comprises an isolated nucleic acid attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, typically DNA oligonucleotides 15 nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

Probes and primers as used in the present invention typically comprise at least 15 nucleotides of the nucleic acid sequences that are shown to encode infection-specific proteins. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 30 or 40 consecutive nucleotides of the disclosed nucleic acid sequences.

Methods for preparing and using probes and primers are well known in the art and are described in, for example Sambrook et al. (1989); Ausubel et al., (1987); and Innis et al., (1990). PCR primer pairs can be derived from a known sequence, for example, by using computer

programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

"Conservative amino acid substitutions" are those substitutions that, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative substitutions.

Original Residue	Conservative Substitution
Ala	Ser
Arg	Lys
Asn	gln, his
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	asn, gln
Ile	leu, val
Leu	ile, val
Lys	arg, gln, glu
Met	leu, ile
Phe	met, leu, tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	trp, phe
Val	ile, leu

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

The substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative, for instance changes in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

"Sequence identity" The similarity between two nucleic acid sequences, or two amino acid sequences is expressed in terms of the level of sequence identity shared between the sequences. Sequence identity is typically expressed in terms of percentage identity; the higher the percentage, the more similar the two sequences are. Variants of naturally occurring infection-specific peptides useful in the present invention are typically characterized by possession of at least 50% sequence identity counted over the full length alignment with the amino acid sequence of a

naturally occurring infection-specific peptide when aligned using BLAST 2.0.1 (Altschul et al., 1997). For comparisons of amino acid sequences of greater than about 30 amino acids, the BLAST 2 analysis is employed using the blastp program set to default perameters (open gap = 11, extension gap = 1 penalty, gap x dropoff = 50, expect = 10, word size = 3, filter on), and using the default BLOSUM62 matrix (gap existence cost = 11, per residue gap cost = 1, lambda ratio = 0.85). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix (gap existence cost = 9, per residue gap cost = 1, lambda ratio = 0.87). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity. The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. It can be accessed at http//www.ncbi.nlm.nih.gov/BLAST/. A description of how to determine sequence identity using this program is available at http://www.ncbi.nlm.nih.gov/BLAST/blast_help.html.

Similarly, when comparing nucleotides, blastn may be used with default settings (rewards for match = 1, penalty for mismatch = -2, open gap = 5, extension gap = 2 penalty, gap x dropoff = 50, expect = 10, word size = 11, filter on), with the default BLOSUM62 matrix (as above). Variants of naturally occurring infection-specific nucleic acid sequences useful in the present invention are typically characterized by possession of at least 50% sequence identity counted over the full length alignment with the nucleic acid sequence of a naturally occurring infection-specific ORF when aligned using BLAST 2.0.1. Useful nucleic acids may show even greater percentage identity, and may, for example, possess at least 55%, at least 65%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity naturally occurring infection-specific ORF.

"Operably linked" A first nucleic acid sequence is "operably" linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Recombinant" A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques.

"Stringent Conditions" Stringent conditions, in the context of nucleic acid hybridization, are sequence-dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5 degrees to 20 degrees lower than the thermal melting point (*Tm*) for the specific sequence at a defined ionic strength and pH. The *Tm* is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook et al. (1989), pages 9.49-9.55. Typical high stringency hybridization conditions (using radiolabeled probes to hybridize to nucleic acids immobilized on a nitrocellulose filter) may include, for example, wash conditions of 0.1 X SSC, 0.5% SDS at a wash temperature of 68°C.

When referring to a probe or primer, the term "specific for (a target sequence)" indicates that the probe or primer hybridizes under high-stringency conditions substantially only to the target sequence in a given sample comprising the target sequence.

"Purified" A purified peptide is a peptide that has been extracted from the cellular environment and separated from substantially all other cellular peptides. As used herein, the term peptide includes peptides, polypeptides and proteins. In certain embodiments, a purified peptide is a preparation in which the subject peptide comprises 50% or more of the protein content of the preparation. For certain uses, such as vaccine preparations, even greater purity may be preferable.

"Immunostimulatory peptide" as used herein refers to a peptide that is capable of stimulating a humoral or antibody-mediated immune response when inoculated into an animal.

"Vaccine" A vaccine is a composition containing at least one immunostimulatory peptide which may be inoculated into an animal with the intention of producing a protective immunological reaction against a certain antigen. The antigen to be protected against may be, for instance, an infectio-specific antigen of *Chlamydia*.

B. ISOLATION OF INFECTION SPECIFIC CHLAMYDIA POLPEPTIDES AND IDENTIFICATION OF GENES ENCODING THESE POLYPEPTIDES

1. ISOLATION OF IncA, IncB AND IncC

Bacterial strains. *Chlamydia* (C. *psittaci* strain GPIC or *C. trachomatis* LGV-434, ser. L2) was cultivated in HeLa 229 cells using standard methods (Caldwell et al., 1981). Purified *Chlamydiae* were obtained using Renografin (E. R. Squibb & Sons, Inc., Princeton, N.J.) density gradient centrifugation. *Escherichia coli* DH5α (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was used as the host strain for transformations with recombinant DNA. *E. coli* XL1-Blue MRF' (Stratagene, La Jolla, Calif.) was used as the host strain for infection with lambda ZAPII phage vector. *E. coli* SOLR (Stratagene) was used as the host strain for infection with *in vivo* excised filamentous lambda ZAPII.

Antisera. MBP (Maltose Binding Protein)-Inc fusion proteins were used as antigens for the production of mono-specific antibody reagents in Hartley strain guinea-pigs. The protein was diluted to 100 $\mu g/ml^{-1}$ sterile saline and mixed with the Ribi Trivalent Adjuvant (Ribi Immunochem.). The antigen/adjuvant emulsion was administered to anaesthetized guinea-pigs using a procedure provided by the manufacturer. Sera were collected 14 days after secondary and tertiary immunizations. Control antisera were produced by immunizing guinea-pigs with adjuvant alone, or with adjuvant plus purified maltose-binding protein.

Convalescent guinea-pig antisera, antisera against live EBs, and antisera against formalin-fixed EBs were produced using standard methods (Rockey and Rosquist, 1994 and Rockey et al., 1995).

***C. psittaci* library construction and screening**. For the *inc*B and *inc*C genes, *C. psittaci* strain GPIC DNA was extracted using a genomic DNA extraction kit (Qiagen) with one modification; dithiothreitol (5mM) was added to the suspension buffer to assist EB lysis. DNA was partially digested with *Tsp*509I and ligated to *Eco*RI digested λ-ZAPII phage arms (Stratagene). The ligation was packaged in vitro with Gigapack extracts according to the manufacturer's instructions (Stratagene). Recombinant phage were plated on *E. coli* XL-1 Blue at densities of approximately 10^4 PFU/150-mm (diameter) plate. Following a nine hour incubation to allow development of the plaques, the plates were sequentially overlaid with nitrocellulose disks and the resulting lifts were processed for immunoblotting with convalescent antisera and antisera to fixed EBs. Of approximately 8,000 plaques, 18 had reactivity with the convalescent sera but not sera generated against EBs. One of these was subcloned into pBluescript SK(-) phagmid by *in vitro* excision in the *E. coli* SOLR strain (Stratagene) and designated pBS200-7.

For the *inc*A gene, genomic DNA from *C. psittaci* strain GPIC was partially digested with *Sau*3A, size-selected (2-8 kb) by electrophoresis through low-melting-temperature agarose, and blunt-ended with T4 DNA polymerase. This DNA was ligated to an *Eco*R1/*Not*1 adapter (Life Technologies), kinased, and ligated to *Eco*R1-digested Lambda ZAP II vector (Stratagene Cloning Systems). Recombinants were packaged (Lambda Gigapack Gold, Stratagene) and used to infect *E. coli* XL1-Blue (Stratagene). Plaques were allowed to develop for 4 h at 37°C. Nitrocellulose filters laden with 10 mM IPTG (US Biochemical Corp.) were placed onto the plaques and incubated for an additional 4 h at 37°C. These filters were removed and placed into a blocking solution consisting of PBS (150 mM NaCl, 10 mM $NaPO_4$, pH7.2) plus 0.1% Tween-20 (TPBS) and 2% BSA-TPBS. Filters were incubated for 1 h, rinsed twice in TPBS, and incubated overnight in convalescent-guinea-pig sera diluted 1:100 in BSA-TPBS. After three washes in TPBS, the filters were incubated for 1 h in ^{125}I-staphylococcal protein A (New England Nuclear) diluted to approx. 124 $nCiml^{-1}$ in BSA-TPBS. Filters were again washed three times in TPBS and positive plaques were detected by exposure of the dried filters to autoradiography film overnight at room temperature. Positive clones were picked and plaque-purified. pBluescript-SK- plasmids

containing the chlamydial genes of interest were recovered from the purified bacteriophage using ExAssist filamentous bacteriophages (Stratagene).

Identification of antigens recognized by convalescent antisera. Recombinant plaques were identified that showed reactivity with convalescent (anti-RB) antisera, but not with anti-EB serum. The purified recombinant phage were converted into pBluescriptII SK plasmid by *in vivo* excision and recircularization and these recombinant DNAs were used to transform *E. coli*. SDS-PAGE and immunoblot analysis of lysates of these recombinant *E. coli* showed that each expressed one or more proteins that reacted with convalescent antisera but not with the EB serum.

DNA Cloning and fusion protein production. The plasmid pJC2 contains a 5.0 kb *Eco*RI GPIC genomic fragment cloned into the pZEro2.1 vector (Invitrogen). To construct pJC2, the *incC* ORF sequence was ^{32}P-radiolabeled using random priming (Gibco-BRL) and used to probe *Eco*RI cut GPIC genomic DNA fragments separated by agarose gel electrophoresis. Fragments in the size range of the positive signal were excised from the gel and purified by Gene-Clean (Bio101). The gel-purified fragments were used in a ligation along with *Eco*RI-digested pZEro2.1. Kanamycin resistant colonies were screened by colony hybridization with radiolabeled *incC*.

MBP fusions of the five ORFs present in pJC2 were produced using the pMAL-C2 vector (New England Biolabs). The reading frame of *incC*, with the exception of the first four codons, was amplified using *Pwo* polymerase (Boehringer Mannheim) and pBS200-7 as the template. The upstream and downstream oligonucleotides for this amplification were

5'-AGAACCGATTTAACTCCAGGCG-3' (SEQ ID NO: 19) and

5'-GCGCGGATCCTTAATGTCCGGTAGGCCTAG-3' (SEQ ID NO: 20), respectively. The vector was digested with *Xmn*I and *Bam*HI, and the amplication product was digested with *Bam*HI. Ligation of these products resulted in an in-frame fusion between the *malE* gene in the vector and the *incC* reading frame from pBS200-7. The stop codon for this construction is provided by the insert. Following ligation, the products were transformed into *E.coli* strain HD5□. The resulting fusion protein (MBP/IncC) was overexpressed and purified by maltose affinity chromatography using an amylose resin supplied by New England Biolabs.

The same approach was used for production of the MBP/IncB fusion protein. The sequence encoding the N-terminal 101 amino acids of the IncB ORF was PCR amplified using the oligonucleotides

5'-ATGTCAACAACACCAGCATCTTC-3' (SEQ ID NO: 21) and

5'-GCGCGGATCCTTAATTAGTGCCTTCTGGATTAGG-3' (SEQ ID NO: 22).

The purified MBP/IncB and MBP/IncC fusion proteins were used as antigen for the production of monospecific antibody in Hartley strain guinea-pigs by standard methods (Rockey et al., 1995). Inserts in each construct were confirmed by DNA sequencing.

For IncA, a maltose-binding protein/IncA fusion protein was produced using the pMAL-C2 vector system from New England Biolabs. The reading frame of *incA* shown in Fig.1, with the exception of the initiator ATG, the *inc*A ORF was amplified using Vent DNA polymerase (New England Biolabs) and plasmid pGP17 as template. The upstream and downstream oligonucleotides for this amplification were

5'-CGCAGTACTGTATCCACAGACAAC-3' (SEQ ID NO: 23) and

5'-GTCGGATCCGAGAAACTCTCCATGCC-3' (SEQ ID NO: 24), respectively. The vector was digested with *Xmn*I and *Bam*HI, and the amplification product was digested with *Sca*I and *Bam*HI. Ligation of these products resulted in an in-frame fusion between the *malE* gene in the vector and the *incA* reading frame from pGP17. The stop codon for this construction is provided by the insert. Following ligation, the products were transformed into *E. coli* strain DH5□. The resulting fusion protein (MBP/IncA) was overexpressed and purified by maltose affinity chromatography using amylose resin (New England Biolabs).

MBP/IncA was used as antigen for the production of mono-specific antibody reagents in Hartley strain guinea-pigs.

DNA sequencing and sequence analysis. The pBS200-7 and pJC2 genomic clones as well as the MBP fusions were sequenced with the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Perkin Elmer/Applied Biosystems Division). Several internal primers were designed to sequence further into the cloned inserts. Sequence assembly was performed using AssemblyLIGN software and sequence analysis was performed with MacVector software (International Biotechnologies Incorporated). Hydrophilicity profiles were determined using the Kyte-Doolittle scale (Kyte and Doolittle, 1982) with a window of 7. Deduced amino acid sequences were compared with the database using the BLAST program (on default settings) available from the National Center for Biotechnology Information on the world wide web. The entire nucleotide sequence of the pJC2 insert was deposited in the GenBank/EMBL Nucleotide Sequence Data Library, under accession number AF017105.

For *incA*, nucleotide sequencing was conducted using the Sequences system (US Biochemical) with the M13 forward and reverse primers, and internal primers synthesized on an Milligen/Biosearch Cyclone Plus DNA synthesizer. Computer analyses were conducted using the MacVector Sequence Analysis Software (International Biotechnologies Incorporated). Hydrophilicity profiles were determined using the Kyte-Doolittle scale (Kyte and Doolittle, 1982) with a window of 7. Secondary-structure predictions were generated using a combination of the Chou-Fasman and Robson-Garnier methods (Robson and Suzuki, 1976; Chou and Fasman, 1978). Deduced amino acid sequences were compared with those in the EMBL and GenBank databases using the BLASTP program available from the National Center for Biotechnology Information.

Electrophoresis and immunoblotting. Polyacrylamide gel electrophoresis (PAGE) was conducted using standard methods (Rockey and Rosquist, 1994). Immunoblotting was performed using standard methods (Rockey et al., 1995).

Immunofluorescence studies. *Chlamydiae* grown in HeLa cells on sterile glass coverslips were fixed for microscopy one of two ways. Cells were either incubated in methanol for 5 minutes, or in the combination fixative periodate-lysine-paraformaldephyde (PLP) for three hours at room temperature followed by permeabilization with 0.05% saponin (Brown and Farquhar, 1989). Immunostaining of the fixed coverslips was performed according to standard methods (Rockey et al., 1995) and visualized under a Nikon Microphot FXA microscope using the 63x objective and oil immersion.

RT-PCR analysis. RNA for RT-PCR analysis was extracted from approximately 2 x 10^{14} *C. psittaci*-infected cells. A Qiagen column was used for extraction and purification according to the manufacturer's instructions (Qiagen). RQ1 RNase DNase (Promega) was used to ensure removal of contaminating genomic DNA. cDNA was prepared by incubating 1.5 µg of RNA, 2.5 µM of the reverse oligonucleotide primer, and AMV reverse transcriptase (Promega) for 1 hour at 42°C in sodium pyrophosphate buffer, according to the manufacturer's instructions. PCR reactions were carried out using 1 µl of the cDNA reaction, 1.25 µM of each oligonucleotide primer, and *Pwo* polymerase (Boehringer Mannheim). Each RT-PCR reaction was accompanied by a positive control reaction that utilized the same primer set and 10 ng of *C. psittaci* genomic DNA, and a negative control reaction in which 1 µl of the same RNA preparation was used as template in the PCR reaction. A control primer set located within the *incC* gene was also used as an RT-PCR control.

Identification of *inc*A, *inc*B and *inc*C genes of *C. trachomatis*. The nucleotide sequence information obtained for the *inc*A, *inc*B and *inc*C of *C. psittaci* (above) was used, with standard methods, to identify the *inc* gene orthologues of *C. trachomatis*. Probes were made that corresponded to the 3' and 5' ends of the *C. psittaci inc* open reading frames. Standard PCR amplification (as above) was used, with the *C. trachomatis* genome as a template, to amplify the corresponding *C. trachomatis* nucleotide sequence. The amplified DNA was then sequenced, using standard methods.

2. ISOLATION OF p242, TroA AND TroB

Bacterial strains. *C. trachomatis* LGV-434, serotype L2, was cultivated in HeLa 229 cells using standard methods (Caldwell et al., 1981). Purified *chlamydiae* were obtained using Renografin (E. R. Squibb & Sons, Inc., Princton, N.J.) density gradient centrifugation (Hackstadt et al., 1992). *Escherichia coli* DH5□ (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was used as the host strain for transformations with recombinant DNA. *E. coli* XL1-Blue MRF' (Stratagene, La Jolla, Calif.) was used as the host strain for infection with lambda ZAPII phage

vector. *E. coli* SOLR (Stratagene) was used as the host strain for infection with *in vivo* excised filamentous lambda ZAPII.

Antisera. Two Cynomolgus monkeys (*Macaca fasicularis*) were anaesthetized and infected urethrally with *C. trachomatis* EBs. Each monkey was infected twice and allowed to recover between infections. Symptoms of infection were monitored over time. Antisera from infected monkeys were tested for reactivity to *Chlamydia* by ELISA (Su et al., 1990).

Sera were collected every two weeks and anti-chlamydial titers were determined. These animals showed mild clinical signs of disease which cleared spontaneously. A second challenge was then administered. Sera were collected from these animals and used to probe a *C. trachomatis* expression library as discussed below. As a control, Guinea Pigs were immunized with killed *C. trachomatis* of the EB form. Sera from these animals were obtained and also used to probe the *C. trachomatis* expression library.

***C. trachomatis* library construction and immunoscreening.** A *C. trachomatis* genomic library was constructed with the lambda ZAPII vector as described above for *C. psittaci*. Approximately 15,000 plaques were plated, transferred to nitrocellulose filters (Schleicher and Schuell, Keene, N.H.) in duplicate, and probed with the monkey convalescent antiserum and with Guinea Pig serum against killed EBs (Bannantine et al., 1998). Plaques that reacted only with the monkey convalescent antisera were selected for further study.

Identification of antigens recognized by convalescent antisera. Four positive recombinant plaques were identified that showed reactivity with convalescent antisera but not with anti-EB serum. The purified recombinant phage were converted into pBluescriptII SK plasmid by *in vivo* excision and recircularization and these recombinant DNAs (pCt1, pCt2, pCt3 and pCt4) were used to transform *E. coli*. SDS-PAGE and immunoblot analysis of lysates of these recombinant *E. coli* showed that each expressed one or more proteins that reacted with convalescent (anti-RB) antisera but not with the anti-EB antiserum. Two of the recombinants clones, pCt2 and pCt3, expressed an identical 19.9 kDa protein (p242). The pCt4 recombinant expressed two different proteins of approximately 32 kDa each that are strongly recognized by convalescent antisera (TroA and TroB).

C. SEQUENCE ANALYSIS

Sequence analysis of pCt1, 2, and 3 revealed overlapping inserts with only one open reading frame (ORF) common in all three. This ORF encodes an approximately 19.9 kDa protein (p242) that shows no similarity to other known proteins. The nucleotide sequence encoding *C. trachomatis* p242, and the amino acid sequence of the protein are shown in SEQ ID NOS:1 and 2, respectively.

The insert in pCt4 contains two complete ORFs which code for two proteins, each of approximately 32kDa (TroA and TroB) that show some homology with proteins from *Treponema*

pallidum. The nucleotide sequences encoding the 32 kDa proteins (TroA and TroB) and the amino acid sequences of these proteins are shown in SEQ ID NOS: 3, 4, 5, and 6.

D. EMBODIMENTS OF THE INVENTION

The present invention includes the nucleotide and amino acid sequences for certain infection-specific proteins from *Chlamydia*. These proteins are p242, TroA, and TroB from *C. trachomatis*, and the IncB, and IncC proteins from *C. psittaci*. The scope of the invention includes fragments of these proteins that may be used in a vaccine preparation or that may be used in a method of detecting *Chlamydia* antibodies. Such fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length, or may even encompass the entire protein.

The present invention also encompasses the use of infection-specific proteins of *Chlamydia*, and the use of nucleotides encoding such proteins. Infection-specific proteins include the IncA, IncB and IncC proteins of *C. psittaci*, the IncA, IncB and IncC proteins of *C. trachomatis*, and the TroA, TroB, and p242 proteins of *C.* trachomatis. The inventors have shown that these proteins are infection-specific by using immunological techniques such as immuno-fluorescence microscopy and immunoblotting.

The present invention includes a vaccine against chlamydial infections comprising infection-specific proteins or fragments of these proteins or proteins that are homologous or show substantial sequence similarity to these proteins. In one embodiment, one or more purified infection-specific proteins may be mixed with a pharmaceutically acceptable excipient to produce a vaccine that stimulates a protective immunological response in an animal. In one embodiment the vaccine may be administered intra-muscularly or sub-cutaneously or intravenously. In another embodiment, the vaccine may be administered by inoculation into or onto the mucous membranes of the subject animal. For example, the vaccine may be administered urethrally or genitally as a liquid or in the form of a pessary. In another embodiment, it may be administered to the mucosa of the lungs as a spray or vapor suspension.

Since at least three amino acids are required to produce an antigenic epitope, the vaccine should comprise at least three consecutive amino acids, preferably at least five consecutive amino acids, and may comprise at least 10, 15, 25, 30, 40, or 45 consecutive amino acids of the infection-specific proteins as shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, and 18.

The vaccine of the invention may be used to inoculate potential animal targets of any of the chlamydial diseases including those caused by *C. psittaci, C. trachomatis, C. pneumoniae* or *C. pecorum*. Indeed the vaccine of the invention may be used to inoculate animals against any disease that shows immunological cross-protection as a result of exposure to infection-specific *Chlamydia* antigen.

Vaccines of the present invention can include effective amounts of immunological adjuvants known to enhance an immune response (e.g., alum). The protein or polypeptide is present in the vaccine in an amount sufficient to induce a protective immune response whether

through humoral or cell mediated pathways or through both. Such a response protects the immunized animal against chlamydial infections specifically by raising an immune response against the Reticulate Body form of *Chlamydia*. Protective antibodies may be elicited by a series of two or three doses of the antigenic vaccine given about two weeks apart.

The present invention also teaches a method of making a vaccine against chlamydial infections. The method of making the vaccine comprises providing a pure (or substantially pure) infection-specific chlamydial peptide or portion thereof, and mixing the peptide with a pharmacologically acceptable excipient or adjuvant. Adjuvants may include commonly used compounds such as alum. Additionally, the vaccines may be formulated using a peptide according to the present invention together with a pharmaceutically acceptable excipient such as water, saline, dextrose and glycerol. The vaccines may also include auxiliary substances such as emulsifying agents and pH buffers. Doses of the vaccine administered will vary depending on the antigenicity of the particular peptide or peptide combination employed in the vaccine and characteristics of the animal or human patient to be vaccinated.

The infection-specific vaccine of the invention is directed towards not only *C. psittaci*, but against all forms of *Chlamydia* including *C. pneumoniae*, *C. trachomatis* and *C. pecorum*, and the vaccine may comprise not just peptides derived from *C. psittaci*, but also orthologous peptides and fragments of such orthologous peptides from other species of *Chlamydia* and peptides that are substantially similar to such peptides.

The present invention also teaches a method of vaccination comprising administering a vaccine formulated as described above to an animal either intravenously, intramuscularly, subcutaneously, by inhalation of a vapor or mist, or by inoculation in the form of a liquid, spray, ointment, pessary or pill into or onto the mucous membranes of the mouth, nose, lungs or urogenital tract or colon.

The methods of the invention may be practiced equally with human or non-human animal subjects.

The present invention also teaches a method of detecting *Chlamydia* infection-specific proteins produced by the Reticulate Body form of the organism. In this embodiment, antibodies raised to the infection-specific proteins are used in an immunological assay such as an Enzyme Linked Immunosorbant Assay or Biotin-Avidin assay or a radioimmunoassay or any other assay wherein specific antibodies are used to recognize a specific protein. Such assays may be used to detect both the quantity of proteins present and also the specificity of binding of such proteins. In such an assay, antibodies have attached to them, usually at the *Fc* portion, a detectable label, such as an enzyme, fluorescent marker, a radioactive marker or a Biotin-Avidin system marker that allows detection. A biological sample is provided from an animal that has been putatively exposed to *Chlamydia*. Such a sample may be, for example, whole blood, serum, tissue, saliva or a mucosal secretion. The sample is then contacted with the labeled antibody and specific binding, if any, is detected. Other methods of using infection-specific antibodies to detect infection-specific

antigens that are present in cells or tissues include immunofluorescense, indirect-immunofluorescense and immunohistochemistry. In immunofluorescense, a fluorescent dye is bound directly to the antibody. In indirect-immunofluorescence, the dye is bound to an anti-immunoglobulin. Specific binding occurs between antigen and bound antibody is detected by virtue of flourescent emissions from the dye moiety. This technique would be particularly useful, for instance, for detection of *Chlamydia* antigen present on a urogenital mucosal smear.

Other techniques, such as competitive inhibition assays may also be used to assay for antigen, and one of ordinary skill in the art will readily appreciate that the precise methods disclosed may be modified or varied without departing from the subject or spirit of the invention taught herein.

The present invention also teaches a method of detection of *Chlamydia* infection-specific antibodies made against the Reticulate Body. In this embodiment a sample is provided from an animal putatively exposed to *Chlamydia* to determine whether the sample contains infection-specific antibodies. Such a sample may be, for example, whole blood, serum, tissue, saliva or a mucosal secretion. This sample is contacted with infection-specific antigens such that the amount and specificity of binding of the antibody may be measured by its binding to a specific antigen. Many techniques are commonly known in the art for the detection and quantification of antigen. Most commonly, the purified antigen will be bound to a substrate, the antibody of the sample will bind via its *Fab* portion to this antigen, the substrate will then be washed and a second, labeled antibody will then be added which will bind to the *Fc* portion of the antibody that is the subject of the assay. The second, labeled antibody will be species specific, i.e., if the serum is from a human, the second, labeled antibody will be anti-human-IgG antibody. The specimen will then be washed and the amount of the second, labeled antibody that has been bound will be detected and quantified by standard methods.

The present invention also teaches a method of treating a *Chlamydial* infection by directing a therapeutic agent against a specific target, such as: (i) an infection-specific protein of *Chlamydia*, (ii) a gene that encodes an infection-specific protein of *Chlamydia* and (iii) an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein said therapeutic agent interacts with said target to affect a reduction in pathology.

For example, the present invention teaches a method of treating chlamydial infection wherein antisense technology is used to prevent the expression of infection-specific genes, thereby preventing the pathologies associated these proteins and preventing reproduction of the RB phase of *Chlamydia*. In this embodiment, RNA molecules complementary to transcripts of infection specific genes are introduced into the host cells that contain *Chlamydia*, and by binding to the mRNA transcripts of the infection-specific genes, prevent translation and therefore expression of the infection-specific proteins that are associated with pathogenesis.

The invention may be practiced to produce a vaccine against any species of *Chlamydia*, including *C. psittaci*, *C. pecorum*, *C. trachomatis* and *C. pneumoniae*.

The following examples illustrate various embodiments of the invention.

EXAMPLE 1: Homologous Sequences

The DNA and protein sequences discussed herein are shown in SEQ ID NOS:1-18. These sequences refer to infection-specific proteins and to the DNA sequences that encode these proteins. Although these sequences are from *C. psittaci* and *C. trachomatis*, it would be equally possible to substitute in the present invention, the orthologs of these sequences from other *Chlamydia* species such as *C. pecorum* and *C. pneumoniae*.

Such orthologous sequences may be obtained from the appropriate organisms by isolation of the genome of the organism, digestion with restriction enzymes, separation of restriction fragments by electrophoresis and purification of these fragments and selection of fragments of appropriate size. Identity of the fragments can be confirmed by dot-blot and by standard DNA sequencing techniques. The orthologous sequences in different *Chlamydia* species may also be found by selection of appropriate PCR primers (selected from appropriate regions flanking the *Chlamydia* gene of interest), and the use of these primers in a PCR reaction, using the genome of the particular species of *Chlamydia* of interest as a template, to amplify the ortholog of interest. Such PCR primers would be selected from the flanking regions to allow specific amplification of the target gene. The fragments so obtained could then be run on a gel to check size and sequenced and compared against the known sequences to determine sequence identity.

The degree of sequence identity between the infection-specific genes of *C. psittaci* or *C. trachomatis* and their orthologs from *C. pecorum* and *C. pneumoniae*, may be determined by comparing sequences using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) as described herein.

Orthologues of interest infection-specific proteins are characterized by possession of at least 50% or greater sequence identity counted over the full length alignment with one of the disclosed amino acid sequences of the *C. psittaci* or *C. trachomatis* infection-specific proteins using gapped blastp set to default parameters (described herein).

EXAMPLE 2: Heterologous Expression of Infection-Specific Antigens

Methods for expressing large amounts of protein from a cloned gene introduced into *Escherichia coli* (*E. coli*) may be utilized for the purification of the *Chlamydia* peptides. Methods and plasmid vectors for producing fusion proteins and intact native proteins in bacteria are well known and are described in Sambrook et al. (1989). Such fusion proteins may be made in large amounts, are relatively simple to purify, and can be used to produce antibodies. Native proteins can be produced in bacteria by placing a strong, regulated promoter and an efficient ribosome binding site upstream of the cloned gene. If low levels of protein are produced, additional steps

may be taken to increase protein production; if high levels of protein are produced, purification is relatively easy.

Often, proteins expressed at high levels are found in insoluble inclusion bodies. Methods for extracting proteins from these aggregates are described in chapter 17 of Sambrook et al. (1989). Vector systems suitable for the expression of *lacZ* fusion genes include the pUC series of vectors (Ruther et al. (1983)), pEX1-3 (Stanley and Luzio (1984)) and pMR100 (Gray et al. (1982)). Vectors suitable for the production of intact native proteins include pKC30 (Shimatake and Rosenberg (1981)), pKK177-3 (Amann and Brosius (1985)) and pET-3 (Studiar and Moffatt (1986)).

Fusion proteins may be isolated from protein gels, lyophilized, ground into a powder and used as antigen preparations.

Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, amphibian or avian species, may also be used for protein expression, as is well known in the art. Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other prokaryotic and eukaryotic cells and cell lines may be appropriate for a variety of purposes, e.g., to provide higher expression, post-translational modification, desirable glycosylation patterns, or other features.

Additionally, peptides, particularly shorter peptides, may be chemically synthesized, avoiding the need for purification from cells or culture media. It is known that peptides as short as 3 amino acids can act as an antigenic determinant and stimulate an immune response. Such peptides may be administered as vaccines in ISCOMs (Immune Stimulatory Complexes) as described by Janeway & Travers, Immunobiology: The Immune System In Health and Disease, 13.21 (Garland Publishing, Inc. New York, 1997). Accordingly, one aspect of the present invention includes small peptides encoded by the nucleic acid molecules disclosed herein. Such peptides include at least 5, and may be at least 10, 15, 20, 25, or 30 or more contiguous amino acids of the polypeptide sequences described herein.

EXAMPLE 3: Production of Antibodies Specific for Infection-Specific Antigens

Antibody against infection-specific antigen is encompassed by the present invention, particularly for the detection of *Chlamydia* infection-specific antigen. Such antibody may be produced by inoculation of an animal such as a guinea-pig or a monkey with infection-specific antigen produced as described above. Such antigen may be a polypeptide as disclosed herein, such as a complete or partial polypeptide from *C. psittaci*, *C. trachomatis*, *C. pneumoniae* or *C. pecorum*. As discussed above, any molecule that can elicit a specific, protective immune response

may be used as a vaccine, but since a minimum of three amino acids are required to do this, a vaccine should comprise at least three amino acids.

The peptide for use in the vaccine of the invention may be naturally derived or may be synthetic such as those synthesized on a commercially available peptide synthesizer. The peptide may also comprise a complete or partial peptide derived from the *C. pneumoniae* or *C. pecorum* infection-specific orthologs of the *C. trachomatis* or *C. psittaci* proteins as set out herein.

In one method of production, a polyclonal antibody is produced by providing a purified peptide which is diluted to 100 micrograms per milliliter in sterile saline and mixed with RiBi Trivalent Adjuvant (RiBi Immunochem Inc). The antigen/adjuvant emulsion is then administered to an anaesthetized guinea pig using a procedure as provided by the manufacturer. Serum is collected 14 days after secondary and tertiary immunizations.

Monoclonal antibody to epitopes of the *Chlamydia* peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler and Milstein (1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected purified protein over a period of a few weeks. The mouse is then sacrificed, and the antibody-producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin, e.g., Hypoxanthene, Aminopterin and Thymidine (HAT) medium. The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Harlow and Lane (1988).

An alternative approach to raising antibodies against the *Chlamydia* peptides is to use synthetic peptides synthesized on a commercially available peptide synthesizer based upon the amino acid sequence of the peptides predicted from nucleotide sequence data.

In another embodiment of the present invention, monoclonal antibodies that recognize a specific *Chlamydia* peptide are produced. Optimally, monoclonal antibodies will be specific to each peptide, i.e., such antibodies recognize and bind one *Chlamydia* peptide and do not substantially recognize or bind to other proteins, including those found in uninfected human cells.

The determination that an antibody specifically detects a particular *Chlamydia* peptide is made by any one of a number of standard immunoassay methods; for instance, the western blotting technique (Sambrook et al., 1989). To determine that a given antibody preparation (for instance from a guinea pig) specifically detects one *Chlamydia* peptide by western blotting, total cellular protein is extracted from a sample of blood from an unexposed subject and from a sample of blood from an exposed subject. As a positive control, total cellular protein is also extracted from

Chlamydia cells grown *in vitro*. These protein preparations are then electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel. Thereafter, the proteins are transferred to a membrane (for example, nitrocellulose) by western blotting, and the antibody preparation is incubated with the membrane. After washing the membrane to remove non-specifically bound antibodies, the presence of specifically bound antibodies is detected by the use of an anti-guinea pig antibody conjugated to an enzyme such as alkaline phosphatase; application of the substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium results in the production of a dense blue compound by immuno-localized alkaline phosphatase. Antibodies which specifically detect the *Chlamydia* protein will, by this technique, be shown to bind to the *Chlamydia*-extracted sample at a particular protein band (which will be localized at a given position on the gel determined by its molecular weight) and to the proteins extracted from the blood of the exposed subject. No significant binding will be detected to proteins from the unexposed subject.

EXAMPLE 4: Use of Infection-Specific Sequences and their Corresponding Peptides and Antibodies in Diagnostic Assays

Another aspect of the present invention is a method for detecting the presence of anti-*Chlamydia* antibodies that react with infection-specific *Chlamydia* proteins, *Chlamydia* peptides and *Chlamydia* nucleic acid sequences in biological samples. These methods include detection of antigen and antibody by ELISA and similar techniques, the detection of proteins in a tissue sample by immunofluorescence and related techniques and the detection of specific DNA sequences by specific hybridization and amplification.

One aspect of the invention is an ELISA that detects anti-*Chlamydia* antibodies in a medical specimen. An immunostimulatory infection-specific *Chlamydia* peptide of the present invention is employed as an antigen and is preferably bound to a solid matrix such as a crosslinked dextran such as SEPHADEX (Pharmacia, Piscataway, NJ), agarose, polystyrene, or the wells of a microtiter plate. The polypeptide is admixed with the specimen, such as blood, and the admixture is incubated for a sufficient time to allow antibodies present in the sample to immunoreact with the polypeptide. The presence of the positive immunoreaction is then determined using an ELISA assay, usually involving the use of an enzyme linked to an anti-immunoglobulin that catalyzes the conversion of a chromogenic substrate.

In one embodiment, the solid support to which the polypeptide is attached is the wall of a microtiter assay plate. After attachment of the polypeptide, any nonspecific binding sites on the microtiter well walls are blocked with a protein such as bovine serum albumin. Excess bovine serum albumin is removed by rinsing and the medical specimen is admixed with the polypeptide in the microtiter wells. After a sufficient incubation time, the microtiter wells are rinsed to remove excess sample and then a solution of a second antibody, capable of detecting human antibodies is added to the wells. This second antibody is typically linked to an enzyme such as peroxidase,

alkaline phosphatase or glucose oxidase. For example, the second antibody may be a peroxidase-labeled goat anti-human antibody. After further incubation, excess amounts of the second antibody are removed by rinsing and a solution containing a substrate for the enzyme label (such as hydrogen peroxide for the peroxidase enzyme) and a color-forming dye precursor, such as o-phenylenediamine is added. The combination of *Chlamydia* peptide (bound to the wall of the well), the human anti-*Chlamydia* antibodies (from the specimen), the enzyme-conjugated anti-human antibody and the color substrate will produce a color that can be read using an instrument that determines optical density, such as a spectrophotometer. These readings can be compared to a negative control such as a sample known to be free of anti-*Chlamydia* antibodies. Positive readings indicate the presence of anti-*Chlamydia* antibodies in the specimen, which in turn indicate a prior exposure of the patient to *Chlamydia*.

In another embodiment, antibodies that specifically recognize a *Chlamydia* peptide encoded by the nucleotide sequences disclosed herein are useful in diagnosing the presence of infection-specific *Chlamydia* antigens in a subject or sample. For example, detection of infection-specific antigens that are present in cells or tissues may be done by immunofluorescence, indirect-immunofluorescense and immunohistochemistry. In immunofluorescense, a fluorescent dye is bound directly to the antibody. In indirect-immunofluorescence, the dye is bound to an anti-immunoglobulin. Specific binding occurs between antigen and bound antibody is detected by virtue of fluorescent emissions from the dye moiety. This technique may be particularly useful, for instance, for detection of *Chlamydia* antigen present on a urogenital mucosal smear. *Chlamydia* may be present in urogenital mucosa, and a smear on a glass slide may be fixed and bathed in a solution containing an antibody specific to the infection-specific antigen. The slide is then washed to remove the unbound antibody, and a fluorescent anti-immunoglobulin antibody is added. The slide is washed again, and viewed microscopically under an appropriate wavelength of light to detect fluorescence. Fluorescence indicates the presence of *Chlamydia* antigen. Alternatively, a urogenital mucosal smear may be taken, the sample cultured with HeLa cells to produce large amounts of the RB form, and immunofluorescence may then be used to detect infection-specific *Chlamydia* antibodies.

Another aspect of the invention includes the use of nucleic acid primers to detect the presence of *Chlamydia* nucleic acids that encode infection-specific antigens in body samples and thus to diagnose infection. In other embodiments, these oligonucleotide primers will comprise at least 15 contiguous nucleotides of a DNA sequence as shown in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, or 17. In other embodiments, such oligonucleotides may comprise at least 20 or at least 25 or more contiguous nucleotides of the aforementioned sequences.

One skilled in the art will appreciate that PCR primers are not required to exactly match the target gene sequence to which they anneal. Therefore, in another embodiment, the oligonucleotides will comprise a sequence of at least 15 nucleotides and preferably at least 20 nucleotides, the oligonucleotide sequence being substantially similar to a DNA sequence set forth

in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, and 17. Such oligonucleotides may share at least about 75%, 85%, 90% or greater sequence identity.

The detection of specific nucleic acid sequences in a sample by polymerase chain reaction amplification (PCR) is discussed in detail in Innis et al., (1990). *PCR Protocols: A Guide to Methods and Applications*, Academic Press: San Diego, part 4 in particular. To detect *Chlamydia* sequences, primers based on the sequences disclosed herein would be synthesized, such that PCR amplification of a sample containing *Chlamydia* DNA would result in an amplified fragment of a predicted size. If necessary, the presence of this fragment following amplification of the sample nucleic acid could be detected by dot blot analysis. PCR amplification employing primers based on the sequences disclosed herein may also be employed to quantify the amounts of *Chlamydia* nucleic acid present in a particular sample (see chapters 8 and 9 of Innis et al., (1990)).

Alternatively, probes based on the nucleic acid sequences described herein may be labeled with suitable labels (such a P^{32} or biotin) and used in hybridization assays to detect the presence of *Chlamydia* nucleic acid in provided samples.

Reverse-transcription PCR using these primers may also be utilized to detect the presence of *Chlamydia* RNA which is indicative of an ongoing infection.

EXAMPLE 5: Production of Chlamydia Vaccines

The purified peptides of the present invention may be used directly as immunogens for vaccination. Methods for using purified peptides as vaccines are well known in the art and are described in Yang et al. (1991), Andersen (1994) and Jardim et al. (1990). As is well known in the art, adjuvants such as alum, Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) may be used in formulations of purified peptides as vaccines. Accordingly, one embodiment of the present invention is a vaccine comprising one or more immunostimulatory *C. trachomatis* or *C. psittaci* peptides encoded by nucleotide sequences as shown in the attached sequence listing, together with a pharmaceutically acceptable adjuvant.

Additionally a vaccine may comprise a defined fraction of the disclosed peptide of *C. trachomatis* or *C. psittaci* or may comprise a peptide wherein the gene coding for the peptide shows substantial similarity to the DNA sequences disclosed herein, such as for orthologous genes of *C. pneumoniae* or *C. pecorum*.

Additionally, the vaccines may be formulated using a peptide according to the present invention together with a pharmaceutically acceptable excipient such as water, saline, dextrose and glycerol. The vaccines may also include auxiliary substances such as emulsifying agents and pH buffers.

It will be appreciated by one of skill in the art that vaccines formulated as described above may be administered in a number of ways including subcutaneous, intra-muscular and intra-venous injection. Doses of the vaccine administered will vary depending on the antigenicity of the particular peptide or peptide combination employed in the vaccine, and characteristics of the

animal or human patient to be vaccinated. While the determination of individual doses will be within the skill of the administering physician, it is anticipated that doses of between 1 microgram and 1 milligram will be employed.

As with many vaccines, the vaccines of the present invention may routinely be administered several times over the course of a number of weeks to ensure that an effective immune response is triggered. Where such multiple doses are administered, they will normally be administered at from two to twelve week intervals, more usually from three to five week intervals. Periodic boosters at intervals of 1-5 years, usually three years, may be desirable to maintain the desired levels of protective immunity.

Alternatively, multiple immunostimulatory peptides may also be administered by expressing the nucleic acids encoding the peptides in a nonpathogenic microorganism, and using this transformed nonpathogenic microorganism as a vaccine.

Finally, a recent development in the field of vaccines is the direct injection of nucleic acid molecules encoding peptide antigens, as described in Janeway & Travers, (1997). Thus, plasmids which include nucleic acid molecules described herein, or which include nucleic acid sequences encoding peptides according to the present invention may be utilized in such DNA vaccination methods.

The vaccine of the invention may be used to inoculate potential animal targets of any of the chlamydial diseases including those caused by *C. trachomatis, C. psittaci, C. pneumoniae* or *C. pecorum*. Indeed the vaccine of the invention may be used to inoculate animals against any disease that shows immunological cross-protection as a result of exposure to infection-specific *Chlamydia* antigen. The protein or polypeptide is present in the vaccine in an amount sufficient to induce a protective immune response whether through humoral or cell mediated pathways or through both. Such a response protects the immunized animal against chlamydial infections specifically by raising an immune response against the Reticulate Body form of *Chlamydia*.

The above embodiments are set out only by way of example and are not intended to be exclusive, one skilled in the art will understand that the invention may be practiced in various additional ways without departing from the subject of the spirit of the invention.

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CLAIMS

What is claimed is:

1. A purified infection-specific protein comprising an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 10,

(e) SEQ ID NO: 12,

(f) an amino acid sequence that differs from an amino acid sequence of (a) to (e) inclusive, by one or more conservative amino acid substitutions, and

(g) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (e) inclusive.

2. An isolated nucleic acid molecule encoding a protein according to claim 1.

3. An isolated nucleic acid molecule according to claim 2 wherein the nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) SEQ ID NO: 1,

(b) SEQ ID NO: 3,

(c) SEQ ID NO: 5,

(d) SEQ ID NO: 9, and

(e) SEQ ID NO: 11.

4. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleotide molecule according to claim 2.

5. A vaccine preparation comprising at least one purified peptide comprising at least 5 contiguous amino acids selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

6. The vaccine preparation of claim 5 wherein the peptide comprises at least 10 contiguous amino acids of at least one of the specified sequences.

7. The vaccine preparation of claim 5 wherein the peptide comprises at least 15 contiguous amino acids of at least one of the specified sequences.

8. The vaccine preparation of claim 5 wherein the purified peptide comprises at least 20 contiguous amino acids of at least one of the specified sequences.

9. A vaccine preparation comprising an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16,

(i) SEQ ID NO: 18,

(j) an amino acid sequence that differs from an amino acid sequence of (a) to (i) inclusive, by one or more conservative amino acid substitutions, and

(k) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (i) inclusive.

10. A method of making a vaccine comprising combining a pharmaceutically acceptable excipient with a purified peptide having an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO:2,

(b) SEQ ID NO:4,

(c) SEQ ID NO:6,

(d) SEQ ID NO:8,

(e) SEQ ID NO:10,

(f) SEQ ID NO:12,

(g) SEQ ID NO:14,

(h) SEQ ID NO:16,

(i) SEQ ID NO:18,

(j) an amino acid sequence that differs from an amino acid sequence of (a) to (i) inclusive, by one or more conservative amino acid substitutions,

(k) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (i) inclusive, and

(l) at least 10 contiguous amino acids from an amino acid sequence of (a) to (i) inclusive.

11. A method of vaccination, comprising administering a vaccine preparation according to claim 5 to a mammal.

12. A method of vaccination, comprising administering a vaccine preparation according to claim 9 to a mammal.

13. A method of detecting an infection-specific *Chlamydia* protein in a biological sample comprising: contacting the biological sample with at least one anti-*Chlamydia* antibody, which antibody is an infection-specific antibody, such that a reaction between the antibody and the infection-specific *Chlamydia* protein gives rise to a detectable effect, and detecting the detectable effect.

14. The method of claim 13 wherein the anti-*Chlamydia* antibody binds specifically to a peptide having an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

15. A method of detecting an infection-specific anti-*Chlamydia* antibody in a biological sample comprising: contacting the biological sample with at least one *Chlamydia* peptide, which peptide is an infection specific peptide, such that a reaction between the peptide and the infection-specific anti-*Chlamydia* antibody gives rise to a detectable effect, and detecting the detectable effect.

16. The method of claim 15 wherein the *Chlamydia* peptide comprises at least 5 contiguous amino acids of a sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

17. The method of claim 15 wherein said *Chlamydia* peptide comprises an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

18. A method of treating a *Chlamydial* infection comprising directing a therapeutic agent against a specific target, said target chosen from the group consisting of: (i) an infection-specific protein of *Chlamydia*, (ii) a gene that encodes an infection-specific protein of *Chlamydia* and (iii) an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein said therapeutic agent interacts with said target to affect a reduction in pathology.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 245-52297	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/08744	International filing date *(day/month/year)* 20 APRIL 1999	Priority date *(day/month/year)* 20 APRIL 1998
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 39/00, 39/118, 49/00; G01N 33/571 and US Cl.: 424/9.2, 184.1, 263.1; 435/7.36		
Applicant THE STATE OF OREGON ACTING BY AND THROUGH THE OREGON STATE BOARD OF HIGHER EDUCATION ON BEHALF OF OREGON STATE UNIVERSITY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of — sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15 NOVEMBER 1999	08 JUNE 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Laurence for RODNEY P. SWARTZ, PH.D. Telephone No. (703)308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/08744

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-26, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ____

[X] the claims:
pages 27-30, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of ____

[X] the drawings:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ____

[X] the sequence listing part of the description:
pages 1-20, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language ENGLISH which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[X] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[X] contained in the international application in printed form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [X] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/08744

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-12 drawn to proteins other than p242, 13-18

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. (See Attached) .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/08744

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-4, 7-12	YES
	Claims	5, 6	NO
Inventive Step (IS)	Claims	1-4, 7-12	YES
	Claims	5, 6	NO
Industrial Applicability (IA)	Claims	1-12	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 5 and 6 lack novelty under PCT Article 33(2) as being anticipated by Dyer et al.

The instant claims are drawn to a composition comprising at least one purified peptide comprising at least 5 contiguous amino acids selected from SEQ ID NO:2. Following a sequence search, it was found that Dyer et al teach a composition which meets the claim limitations, i.e., a peptide composition comprising ≥5 contiguous amino acids selected from SEQ ID NO:2 (Figure 1).

Claims 1-4 and 7-12 drawn to the p242 protein as exemplified by the amino acid sequence SEQ IDNO:2 and the nucleic acid sequence SEQ ID NO:1 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the purified protein p242, or vaccine preparations where the peptide comprises ≥15 contiguous amino acids of SEQ ID NO:2.

---------- NEW CITATIONS ----------

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

I. BASIS OF REPORT:

5. (Some) amendments are considered to go beyond the disclosure as filed:
NONE

III. NON-ESTABLISHMENT OF REPORT:

No international search report has been established for claim numbers claims 1-12 drawn to other than p242 protein, 13-18.